THERMOPHILIC AND MESOPHILIC AMINOPEPTIDASES FROM BACILLUS STEAROTHERMOPHILUS

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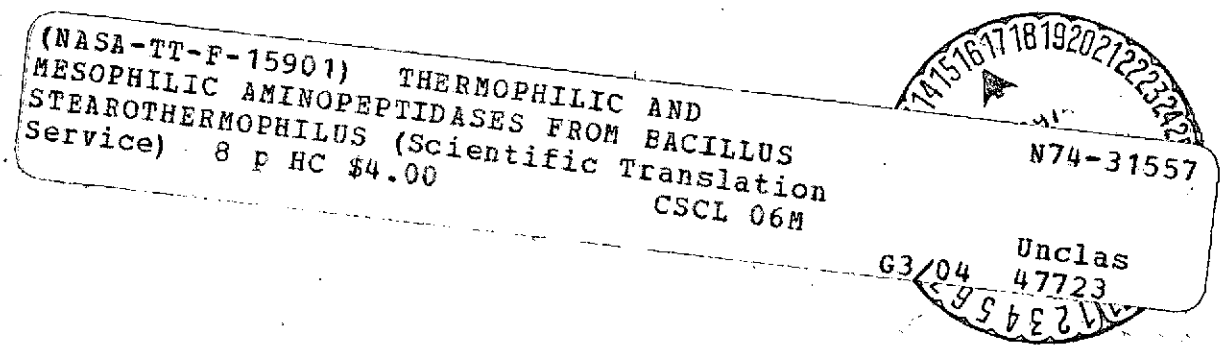
Translation of "Thermophile und mesophile Aminopeptidasen aus Bacillus stearothermophilus", Angewandte Chemie, Vol. 79, No. 20, 1967, pp. 906 - 907.



NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D. C. 20546 AUGUST 1974

STANDARD TITLE PAGE

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
NASA TT F-15,901		

4. Title and Subtitle	5. Report Date
Thermophilic and mesophilic aminopeptidases from Bacillus stearothermophilus	August 1974
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
H. Zuber and G. Roncari	10. Work Unit No.
	11. Contract or Grant No. NASw-2483

9. Performing Organization Name and Address	13. Type of Report and Period Covered
SCITRAN Box 5456 Santa Barbara, CA 93108	Translation
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration Washington, D.C. 20546	14. Sponsoring Agency Code

15. Supplementary Notes

Translation of "Thermophile und mesophile Aminopeptidasen aus Bacillus stearothermophilus". Angewandte Chemie, Vol. 79, No. 20, 1967, pp. 906 - 907.

16. Abstract

Various strains of B. stearothermophilus contain different proportions of three aminopeptidases. Obligately thermophilic strains contain more of the thermophilic enzyme; obligately mesophilic strains contain very little of it, and facultative strains contain similar amounts of the three.

17. Key Words (Selected by Author(s))	18. Distribution Statement
	Unclassified - Unlimited

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	6	

THERMOPHILIC AND MESOPHILIC AMINOPEPTIDASES FROM BACILLUS STEAROTHERMOPHILUS [1]

H. Zuber and G. Roncari*

We have been able to isolate three aminopeptidases, /906** differing in molecular weight, thermal stability, and substrate specificity, from several strains of Bacillus stearothermophilus [2]. Aminopeptidase I (AP I) is thermostable and is, therefore, designated as the thermophilic enzyme. Aminopeptidases II and III (AP II and AP III) are mesophilic enzymes, and thermolabile. All three enzymes are metalloenzymes. In the native form, they may exist as Co^{2+} or Mn^{2+} enzymes, as Co^{2+} and Mn^{2+} reactivate the enzymes best after inactivation with EDTA ($Co^{2+} = Mn^{2+} > Mg^{2+} > Ni^{2+} > Cd^{2+}$). Ca^{2+}, Zn^{2+} and Fe^{2+} do not activate, but inhibit at concentrations from 0.001 to 0.05 M. The pH optimum is at pH = 7.5 to 9 for all three enzymes. The substrate specificity (Table 2) is in part sharply different from the specificity of the leucine aminopeptidase (LAP) [3] from hog kidneys.

AP I has a specific activity of 1,000 units $mg^{-1} \cdot min^{-1}$, if we define the unit as hydrolysis of 1 µmol of substrate per minute. The molecular activity is 150,000 mol. (mol Enzyme^{-1}) $\cdot$ min^{-1}. Both activities were determined by hydrolysis of a 0.045 M solution of Gly-Leu-Tyr in 0.05 M Tris-HCl buffer, pH=7.2, 10^{-3} M Co^{2+} at 65° C.

AP I was purified by repeated chromatography in 0.05 M Tris-HCl buffer (10^{-3} M Co^{2+}) on Sephadex G-150 and DEAE-Sephadex A-50. AP II and AP III were purified by repeated chromatography on Sephadex G-100 and DEAE-Sephadex. AP I,

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** Numbers in margin indicate pagination in original foreign text.

Table 1. PROPERTIES OF THE AMINOPEPTIDASES I - III

	AP I	AP II	AP III
Molecular weight [a]	[ca. 150000]	[ca. 70000]	[ca. 80000]
Michaelis constant, K_m (60°C, Leu-Gly)(mmol/l)	[95]	[37]	[4]
Activation energy [b] (kcal/mole)	[16,3]	[16,3]	
Optimum temperature [b] (°C)	[90]	[60]	[60]
Thermostability [c] (Percent of the initial activity after			
3 min		[64]	[81]
18 min	[120]	[35]	[54]
120 min	[120]	[0]	[0]
at 80°C			

[a] Determined by gel filtration with Sephadex G-200, 0.05 M Tris-HCL buffer, pH = 7.2, 10^{-3} M Co^{2+}.

[b] Determined through hydrolysis of a 0.0075 - 0.06 M solution of Leu-Gly (AP I and AP II) or a 0.0002 - 0.002 M solution of Leu-Gly (AP III) (0.05 M Tris-HCl buffer, pH = 7.2, 10^{-3} M Co^{2+}; incubation time: 10 minutes at 20, 40, 60, 80, 90 °C).

[c] Determined by hydrolysis of a 0.06 M Leu-Gly solution, 0.05 M Tris-HCl buffer, pH = 7.2, 10^{-3} M Co^{2+}; incubation time: 10 minutes at 65°C (AP I) or 37°C (AP II and AP III).

AP II and AP III are homogeneous in polyacrylamide gel electrophoresis [4] (pH = 8.9, 45 minutes). The first experiments in the ultracentrifuge yielded for AP I: $S_{20} = 2.80 \cdot 10^{-13}$ and $D_{20} = 1.8 \cdot 10^{-7} \pm 0.16$ (symmetric bands).

Table 2. SUBSTRATE SPECIFICITIES

(Substrate concentration: 0.045 M, 65°C, 0.05 M Tris-HCl buffer, pH = 7.2, 0.001 M Co^{2+}). The numbers, except for column C, indicate percent hydrolysis in 5 minutes.

Peptide	AP I	C_1 [a]	AP II	AP III
Leu-Gly	16	30	1,6	1,6
Gly-Leu	0,8	1,4	0,1	0,09
Leu-amid	5	8,8	9	1,2
Leu-Gly-Gly	30	50	25	80
Gly-Leu-Tyr	36	77	8	18
Pro-Tyr-Lys	3,2	5,6	2,7	25
Glu-Ala-Ala	5,5	9,8	1,2	1,7
Lys-Tyr-Glu	1,5	2,6	1,3	24

[a] $C_1 = k_1/E$: k_1 = first order reaction rate; E = protein concentration (mg N/ml), assuming 15% nitrogen in the protein.

From this it appears that AP I exists in solution as a severely hydrolysed or very extended molecule. AP I is stable in 8 M urea solution, but is inactivated and split into fragments by reagents which break hydrogen bonds, such as formic acid or LiBr.

All three enzymes are found as early as the beginning of the log phase in the bacterial growth curve. They appear both in the cells and in the culture medium. The yield of the enzyme is maximal at the end of the log phase and in the stationary phase (24-30 hours). AP I, AP II and AP III are present in different amounts in the different strains of Bacillus stearothermophilus. Obligately thermophilic strains (temperature optimum at 55°C, no growth at 37°C) produce much AP I and AP II and little AP III, while obligately mesophilic strains

(temperature optimum 37°C, no growth at 55°C) contain much AP III and very little AP I and AP II. Facultative strains (growth at 37°C and 55°C) can contain the three aminopeptidases in comparable amounts.

In order to obtain the crude enzyme mixture, the bacteria were disrupted with ultrasound or in the sand mill. Lysozyme treatment liberates only AP II and AP III. AP I appears to be bound more strongly to the cell membranes. The aminopeptidases were obtained from the culture medium by adsorption on DEAE-Sephadex (pH $\doteq$ 7.2) and elution with 1 M NaCl solution (0.05 M Tris-HCl buffer, pH = 7.2, 10^{-3} M Co^{2+}).

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1. This work was supported by the Swiss National Fund for Promotion of Scientific Research (Project No. 3426).

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Translated for National Aeronautics and Space Administration under contract No. NASw 2483, by SCITRAN, P. O. Box 5456, Santa Barbara, California, 93108.